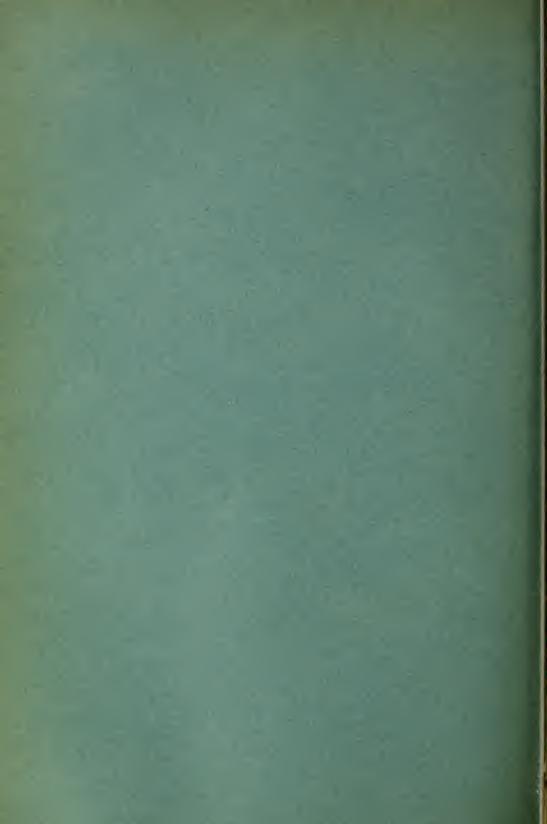
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# The Immunizing Value of Commercial Vaccines and Bacterins Against Hemorrhagic Septicemia

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### THE IMMUNIZING VALUE OF COMMERCIAL VACCINES AND BACTERINS AGAINST HEMORRHAGIC SEPTICEMIA

By L. VAN ES AND H. M. MARTIN

In the course of certain studies having to do with hemorrhagic septicemia, we were particularly struck with the difficulties which we encountered in the immunization of susceptible laboratory animals (rabbits). Aside from a rather transitory and very unstable immunity secured by the use of an anti-serum prepared by us, there was little or no evidence that injections of bacterins or vaccines, as made in our laboratory, were followed by any degree of resistance against a trial injection of B. bipolaris.

This difficulty in no small degree excited our curiosity regarding the immunizing value of the considerable number of "Hemorrhagic Septicemia bacterins and vaccines" which during recent years have become such a prominent article of commerce. Most of those preparations appear to be based upon a belief that by heating or by other means of killing or attenuation of the B. bipolaris a dependable immunizing agent can be produced, although we are not aware that this has been proven to be the case by exact laboratory experiments. In addition we have seen no evidence that the immunizing value of the products mentioned is being controlled by any standard method by either the manufacturer or the user. Considerations of this kind, however, do not appear to have a restrictive influence on the sale of those substances and we are certain that the amount of money annually expended on them by the raisers of farm animals is quite a considerable one.

In the hope of securing some information relative to the immunizing value of the agents mentioned, we undertook a series of experiments in which the immunity of the bacterin and vaccine injected laboratory animals was definitely tested by subsequent inoculations with B. bipolaris.

In nearly all the cases, the vaccines or bacterius were purchased directly from the manufacturer, selecting those especially which were most conspicuously advertised. The degree of immunity produced by them was always tested by the use of B. bipolaris cultures or material primarily obtained from the same species as the one upon which the bacterin or vaccine was supposed to confer protection. In one instance we were even fortunate enough to test the immunity of our test animals against the very strain of B. bipolaris found in the preparation.

In the matter of dosage, we have with but few exceptions selected the one recommended on the label. In some cases we used smaller, in others larger doses in order to ascertain a possible influence upon immunity by such variations.

In only a few instances did the vaccine or bacterin injections produce any immediate unfavorable effect on the test rabbits. In most of those cases there was indisposition or even sudden deaths immediately following the injection and this we could attribute entirely to the presence of preservative agents. With those few exceptions we encountered no trouble caused by the relative large doses.

### Sample No. 113.

The first preparation which we had occasion to give a trial was one sold as a "Mixed Infection Bacterin." This name, at first sight, made it appear that it would perhaps be unwise to expect an immunity against hemorrhagic septicemia from its use. However, in accompanying printed matter the manufacturer assured us that it contained forty-five per cent of B. suisepticus, and because of the further statement that "Each two mill contains the most satisfactory combinations of organisms and in amounts sufficient to insure immunity against each possible infection," we considered ourselves justified in using this bacterin in our tests. In passing it may be of interest to observe that according to the manufacturer's claims it should cause immunity against four other bacterial species aside from hemorrhagic septicemia.

To what extent any claims in regard to the latter disease are sustained by our experiments may be seen from Table I.

TABLE I

Rabbit	infection No.	113	Injectio loopful o B. bir	of culture olaris	Date of	Remarks
No.	Quantity	1/14 Quantity	1/14	1/27	deaths	
	c.c.	c.c.				
1	.5 .5 .5 .5 .5 1 1 2 2 2	2 2      	X X X X X X X X X	X X X X X X X X X X X X X X X X X X X	1/29 1/28 1/15 1/15 1/15 1/15 1/15 1/15 1/15 1/1	Control I Control II

NOTE—The fact that an injection was made is indicated by the X. A dash (—) is used to show that no injection took place.

It will be observed that two of the three rabbits which received the l c.c. doses actually survived. Therefore it was thought wise to make some further tests with the same dose. What happened to the rabbits so treated is shown in Table II.

TABLE II

Rabbit No.	Commercial mixed infection bacterin No. 113 1/17 Quantity c.c.	Injection of one loopful of culture B. bipolaris No. 59	Date of deaths	Remarks
1 2 3 4 5	1 1 1 1 1	X X X X X	1/28 1/28 1/28 1/28 1/28 1/28	

Sample No. 148.

The material used in this test reached the laboratory under the label: "Hemorrhagic Septicemia Vaccine, (Avian)." The detailed account of the experiment is given in Table III.

TABLE III

Rabbit	Fowl cholera vaccine No. 148 2/3 Quantity 1 c.c.	One loopful culture B. bipolaris avisepticus 2/11 Strain 38 B.	Date of deaths	Remarks
1	— X X X X X X X X X X X X X X X X X X X	• X X X X X X X X X X X X X X X X X X X	2/12 2/12 2/12 2/12 2/12 2/12 2/12 2/12	All injections were made intravenously.

### Sample No. 176

A preparation sold as "Hemorrhagic Septicemia Bacterin for Cattle." The test gave the results shown in Table IV.

TABLE IV

Rabbit	bact No.	nercial terin 176 e.c.	Injection of one loopful of culture B. bipolaris No. 156 2/28	Dates of deaths	Remarks
1 2 3	$\frac{-}{x}$	$\frac{-}{x}$	X X X	3/1 3/1 3/1 3/10	Subject in poor condition.  Survived injection of strain 156, but when injected3/8 with strain 59 it died two days later.
5 6	X X	X X	X X	3/1 3/10	Survived injection of strain 156, but when injected on
7 8 9 10	X X X X	X X X X	X X X X	3/2 3/4 3/3 3/4	3/8 with strain 59 it died two days later.

### Sample No. 359.

This preparation was sold to us as "Hemorrhagic Septicemia Combined Bacterin (Bovine)." The immunity of the rabbits treated with this substance was tested with three strains of B. bipolaris of bovine origin. It will be noted that the virulence of one of those strains was lost and that the rabbits with one exception succumbed to a subsequent infection. For details of this experiment see Tables V, VI and VII.

TABLE V

Rabbit	Commercial bacterin No. 359 2 c.c. 6/18	Injection of one loopful of virulent blood B. bipolaris No. 172 7/1	Injection of one loopful of virulent blood B. bipolaris No. 214 7/12	Dates of deaths	Remarks
1	X	X	_	7/12	Subject was sick one week before death.
2 3 4 5 6	X X X X X	X X X X X		7/2 7/5 7/5 7/2 7/2	( Sick for several days.
7	X	X	_	7/25	Marked pneumonia. No organisms in heart's blood.
89	<u>X</u>	X X	_	7/2 7/4	

TABLE VI

Rabbit No.	Commercial bacterin No. 359 2 c.c. 6/18		Injection of one loopful of virulent blood (B. bipolaris) No. 214 7/12	Dates of deaths	Remarks
1 2 3	X X X	X X X	=	7/2 7/2 7/7	(Was sick for three
4	X	Х .	_	7/16	days previous to 7/12. B. bipolaris in heart blood.
5 6 7 8 9 10 11	X X X X X X X	X X X X X X		7/2 7/2 7/2 7/2 7/2 7/2 7/2 7/8	

TABLE VII

Rabbit No.	Commercial bacterin No. 359 2 c.c. 6/18	Injection of one loopful of virulent blood (B. bipolaris) No. 307 7/1	Injection of one loopful of virulent blood (B. bipolaris) No. 214 7/12	Dates of deaths	Remarks
1 2 3 4 5	X X X X X	X X X X	X X X —	7/13 	{ Pleuro pneumonia B. bipolaris in blood.
6 7 8 9 10 11	X X X X —	X X X X X X	$\frac{x}{x}$ $\frac{x}{x}$	7/13 7/13 7/4 7/13 7/3 7/13 7/13	

With the same combined bacterin we furthermore treated three rabbits not less than eight times at forty-eight hour intervals. The results are given in Table VIII.

TABLE VIII

Rabbit No.	Received 2 c.c. of commercial bacterin No. 359 on the following dates: 6/18 6/28 6/20 6/30 6/23 7/1 6/27 7/3	Injection of one loopful of virulent blood (B. bipolaris) No. 214 7/9	Injection of one loopful of virulent blood (B. bipolaris) No. 172 7/12	Dates of deaths	Remarks
1 2 3 4 5	X X -	X X X X	<u>x</u> _ _ _	7/13 7/10 7/10 7/10 7/10 7/10	

Sample No. 485.

A product sold as "Bacillus Suisepticus Bacterin (for swine plague)."

As the manufacturer of this material in common with others, indicates in his advertising matter that repeated bacterin injections are desirable, we designed this series of experiments so that three groups of six rabbits each received respectively one, two and three bacterin injections while another group of ten rabbits were injected not less than twelve times. All injections were made every other day and were so timed that the entire series received its last immunizing dose on the same day.

Ten days later each of the rabbits in this series received an inoculation with 1/10 c.c. of virus mixture prepared by taking the heart blood of a rabbit dead as a result of inoculation with B. bipolaris (from swine) mixed with twenty parts of salt solution and added to equal parts of a 24-hour boullion culture of the same organism. Table IX shows the results of those trials.

Romarks	TVCAIRGA IND	(Died $2\frac{1}{2}$ hours after injection.  (B. bipolaris in heart blood.  Died $2\frac{1}{2}$ hours after injection.  Dead $2\frac{1}{2}$ hours after injection.
Dates of	deaths	100/26 10
Injections B. bipolaris No. 49	1-10 c.c. 10-25	**************************************
	10-15	
	10-11 10-13 10-15	
ಲೆ	10-11	
is 2 c	10-9	
ction	10-7	**************************************
inje	10-5	
No. 485 Bacterin injections 2 c.c.	10-3	
5 Bac	10-1	
. 48	9-29	**************************************
N	9-27	****
	9-25	
	9-23	
Rabbit	No.	12.8.4.0.0c.0011212147011222222222222222222222222222

SAMPLE NO. 549.

latter were virulent to rabbits, in spite of the previous injections, there was no opportunity of testing any immunity which may be obtained by this method of treatment. In the final tabulation of our results the data of Table X were not used. injections are required. The first with killed organisms and the second with living ones. As the The material tested under this number varies from others tested in as much as two distinct

TABLE >

Remarks	Dead four hours after vaccine injection.  (When killed subject had large abscesses in flank and hind quarter.  (X) Only 2 c.c. of bacterin.  Control. Heart blood swarming with B. bipolaris.  (Control. Dead six hours after injection.  Control. Dead six hours after injection.
Dates of deaths	12/6   12/6
Injection B. bipolaris No. 549	12/16 
Vaccine No. 549 Injection 4 c.c.	XXXXX
Bacterin No. 54.9 Injection 4 c.c 11/25	XXXXXX XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
Rabbit No.	100 100 100 100 100 100 100 100 100 100

SAMPLE NO. 620.

the fact that this vaccine was virulent to many rabbits of the series as eight died after the first injection and four after the second one. Trom those rabbits we isolated the B. bipolaris and this was the vaccine injected. Table XI shows, however, that even this failed to protect them against the very virus from which the vaccine was made. The table further shows that one of those surviving An interesting feature of this experiment is used to test the immunity of the survivors. Of the latter several had shown sickness as a result of rabbits (26) which had received and survived a 10 c.c. injection succumbed to subsequent infection A "Hemorrhagic Septicemia Vaccine for cattle." with the organism isolated from the vaccine.

## TABLE XI

Remarks		Subject died a few hours after in- oculation. Heart blood was swarm-	Heart blood swarming with B.bipolaris.	Heart blood swarming with B.bipolaris.							Heart blood swarming with B.bipolaris.		/ Heart blood swarming with B. bi-	( polaris.
Dates of deaths	2/11	2/14	2/14	2/12	2/22	2/22	2/22	2/11	2/22	2/22	2/13	2/22	2/22	2/17
Injection B. bipolaris No. 620					-	1	1		-	-	1	1	-	1
Injection B. bipolaris No. 620	2/21				2/21	2/21	2/21	1	2/21	2/21	and controlled in	2/21	2/21	
Injection vaccine No. 620 2/28					-		1	- Paragonal	- Investment	1		•	1.	-
Injection vaccine No. 620 2/14	2 c.c.	2 c.c.	- 6		2 c.c.	2 c.c.	2 c.c.		4 c.c.	4 c.c.	1	4 c.c.	4 c.c.	4 c.c.
Injection vaccine No. $620$	2 c.c. 2 c.c.	2 c.c.	2 c.c.	2 C. C.	2 c.c.	2 c.c.	2 c.c.	2 c.c.	4 c.c.	4 c.c.	4 c.c.	4 c.c.	4 c.c.	4 c.c.
Rabbit No.	$\frac{1}{2}$	3	٠٠٠٠٠	6	7	8	9	0	1	22	3	4	5	6

Remarks	Heart blood swarming with B. bipolaris.  Heart blood swarming with B. bipolaris.  Subject drowsy after last injection. Heart B. swarming with B. bipolaris. Developed apparent anaphylactic symptoms after last injection from which it recovered in 15 min. Heart blood swarming with B. bipolaris. Slight disturbance after injection of 2/2s. Purulent pericarditis on which abundance of B. bipolaris. Control Control
Dates of deaths	222222222 2 2 2222 4222211112 2 1 2222 4222211116 2 1 2222 422222 2 2 2222
Injection B. bipolaris No. 620	3/11
Injection B. bipolaris No. 620	2 / 21 2 / 21 2 / 21 3 / 9
Injection vaccine No. 620 2/28	
Injection vaccine No. 620 2/14	44 c.c. 14 c.c. 1 c.c. 1 c.c.
Injection vaccine No. 620 2/9	44444444444444444444444444444444444444
Rabbit No.	17. 18. 19. 22. 22. 22. 24. 25. 27. 27. 30. 30.

Sample No. 621.

This preparation was called on the label "Hemorrhagic Septicemia Vaccine for Swine." The results of our tests are shown in Table XII.

TABLE XII

Rabbit	Vaco	cine injoint No. 62 $\frac{2}{15}$		Injection B. bipolaris No. 59	Dates of deaths	Remarks	
1	2 c.c. 2 c.c. 2 c.c. 2 c.c. 2 c.c. 2 c.c. 2 c.c. 4 c.c. 4 c.c. 4 c.c. 4 c.c. 4 c.c. 4 c.c. 5 c.c. 1 c.c.	2 c.c. 2 c.c. 2 c.c. 2 c.c. 2 c.c. 2 c.c. 2 c.c. 2 c.c. 4 c.c. 4 c.c. 4 c.c. 4 c.c. 4 c.c. 4 c.c. 1 c.c.	10 c.c.	2/24 2/24 2/24 2/24 2/24 2/24 2/24 2/24	2/25 2/25 2/25 2/25 2/25 2/25 2/25 2/25	{ Heart blood contained many B. bipolaris.  Control Control Control Control Control Control Control	

Sample No. 717.

A specimen of "Hemorrhagic Septicemia Bacterin (Bovine)." The details of the tests made with this material are given in Table XIII.

TABLE XIII

Rabbit	Injecti	ions bac 5 c	eterin N	o. 717	Injections B. bipolaris No. 738 Loopful	Dates of deaths	Remarks
	3/31	4/3	4/6	4/9	4/19		
1	X X X X X X X X X X X X X X X X X X X	X X X X X X X X X X X X X X X X X X X	X X X X X X X X X X X X X X X X X X X	X X X X X X X X X X X X X X X X X X X	1/10 1/10 1/10 1/10 1/4 1/4 1/4 1/4 1/4 1/4 1/4 1/4 1/4 1/4	4/20 4/20 4/20 4/20 4/20 4/20 4/20 4/20	Control Control

Sample No. 720.

This preparation is sold as "Hemorrhagic Septicemia Vaccine (Bovine)." The experiments made with this substance are given in Table XIV.

TABLE XIV

Rabbit	Injections vaccine No. 720 4 c.c.  4/3 4/6 4/9		Injections B. bipolaris No. 738 Loopful 4/19	Dates of deaths	Remarks	
1 2 3 4 5 6 7 8 9	X X X X X X X X X	X X X X X X X X X	X X X X X X X X X	1/10 1/10 1/10 1/4 1/4 1/4 1/4 1/4 1/4 1/4	$\begin{array}{c} 4/20 \\ 4/20 \\ 4/20 \\ 4/20 \\ 4/20 \\ 4/20 \\ 4/20 \\ 4/20 \\ 4/20 \\ 4/20 \\ 4/20 \end{array}$	$\left\{ egin{array}{ll}  ext{Received more than 4} \\  ext{c.c. vaccine 4/3} \end{array} \right.$
11	X X X X X X X	X X X X X X X X	X X X X X X X	1/4 1/4 1/4 1/4 1/4 1/4 1/10 1/4	$\begin{array}{c} 4/20 \\ 4/20 \\ 4/20 \\ 4/20 \\ 4/20 \\ 4/20 \\ 4/20 \\ 4/20 \\ 4/20 \\ 4/20 \end{array}$	Control Control

Sample No. 735.

This material was sold to us under the label "Swine Plague Bacterin." Table XV relates the tests made in detail.

TABLE XV

Rabbit	Injections bacterin No. 735 2 c.c.			Injections B. bipolaris No. 59 Loopful		Dates of deaths	Remarks
2,0,	4/13	4/16	4/20	4/28	5/11	deaths	
1	X X X X X X X X X X X X X X X X X X X	X X X X X X X X X X X X X X X X X X X	X X X X X X X X X X X X X X X X X X X	1/50 1/50 1/50 1/50 1/50 1/50 1/50 1/10 1/50 1/5	1/10	5/12 4/29 4/29 4/29 4/29 4/29 4/29 4/29 4/30 4/30 4/29 4/29 4/29 4/29	Pneumonia, pericar-
16	X	X	X	1/50	1/10	5/19	ditis. Organs contained B. bipolaris.
17	X X X X —	X X X X —	X X X 	1/50 1/50 1/50 1/50 1/50 1/50		4/29 4/29 4/29 4/29 4/29 4/29	Control Control

The results of the entire series of experiments as far as the acquisition of immunity on the part of the laboratory animals is concerned are summarized in Table XVI.

### TABLE XVI

No. rabbits treated	No. of bacterin	Res	ults	Remarks .
and immunity tested	or vaccine injections	Rabbits dead	Rabbits alive	Itemarks
59. 47. 48. 20. 3. 10. 187	1 2 3 4 8 12	56 47 48 20 3 10 184	3 0 0 0 0 0 0 3	Only those rabbits which lived long enough to receive the final virus dose were considered in the construction of this table.

### CONCLUSIONS

It does not appear that the vaccines and bacterins experimented with have any antigenic value which expresses itself in actual resistance to B. bipolaris infection. We encountered uniform negative results, no matter if we injected those substances once or a dozen times; a virus injection was always certain to kill the animals.

The indications are that if hemorrhagic septicemia is in reality a frequent and very fatal disease to our farm animals, that the commercial immunizing agents against it are indeed a poor staff to lean on and that if those diseases are at all to be combatted in a serious manner, we will have to look to other methods for relief.

